



TITLE:

# The effects of a 4-week static stretching programme on the individual muscles comprising the hamstrings

AUTHOR(S):

Ichihashi, Noriaki; Umegaki, Hiroki; Ikezoe, Tome; Nakamura, Masatoshi; Nishishita, Satoru; Fujita, Kosuke; Umehara, Jun; Nakao, Sayaka; Ibuki, Satoko

---

CITATION:

Ichihashi, Noriaki ...[et al]. The effects of a 4-week static stretching programme on the individual muscles comprising the hamstrings. Journal of sports sciences 2016, 34(23): 2155-2159

ISSUE DATE:

2016-12

URL:

<http://hdl.handle.net/2433/230932>

RIGHT:

This is an Accepted Manuscript of an article published by Taylor & Francis in Journal of Sports Sciences on 2016, available online: <http://www.tandfonline.com/10.1080/02640414.2016.1172725>; The full-text file will be made open to the public on 1 June 2018 in accordance with publisher's 'Terms and Conditions for Self-Archiving'; この論文は出版社版ではありません。引用の際には出版社版をご確認ご利用ください。; This is not the published version. Please cite only the published version.

1    **Title page**

2    **Title : The effects of a 4-week static stretching program on the**  
3    **individual muscles comprising the hamstrings**

4    **Running title: Effect of 4-week stretching on muscle hardness**

5    Noriaki Ichihashi<sup>a</sup>, Hiroki Umegaki<sup>a</sup>, Tome Ikezoe<sup>a</sup>, Masatoshi Nakamura<sup>b</sup>, Satoru

6    Nishishita<sup>c</sup>, Kosuke Fujita<sup>a</sup>, Jun Umehara<sup>a</sup>, Sayaka Nakao<sup>a</sup>, Satoko Ibuki<sup>a</sup>.

7    *Affiliations:*

8    Noriaki Ichihashi    \*Corresponding author

9    <sup>a)</sup> Human Health Sciences, Graduate School of Medicine, Kyoto University,

10    53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.

11    Telephone: +81-75-751-3951

12    E-mail: [ichihashi.noriaki.5z@kyoto-u.ac.jp](mailto:ichihashi.noriaki.5z@kyoto-u.ac.jp)

13    Hiroki Umegaki

14    <sup>a)</sup> Human Health Sciences, Graduate School of Medicine, Kyoto University,

- 15 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.
- 16 Telephone: +81-75-751-3951
- 17 Email: [pontajilansa@yahoo.co.jp](mailto:pontajilansa@yahoo.co.jp)
- 18 Tome Ikezoe
- 19 <sup>a)</sup> Human Health Sciences, Graduate School of Medicine, Kyoto University,
- 20 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.
- 21 Telephone: +81-75-751-3967
- 22 Email: [ikezoe.tome.4u@kyoto-u.ac.jp](mailto:ikezoe.tome.4u@kyoto-u.ac.jp)
- 23 Masatoshi Nakamura
- 24 <sup>b)</sup> Faculty of Health and Sports Science, Doshisha University,
- 25 1-3 Tatara Miyakodani, Kyotanabe, Kyoto 610-0394 Japan
- 26 Telephone: +81-75-751-3935
- 27 E-mail: [manakamu@mail.doshisha.ac.jp](mailto:manakamu@mail.doshisha.ac.jp)
- 28 Satoru Nishishita
- 29 <sup>c)</sup> Institute of Rehabilitation Science, Tokuyukai Medical Corporation,

30 3-11-1 Sakuranomachi, Toyonaka, Osaka 560-0054 Japan

31 Telephone: +81-6-6857-7758

32 Email: [satoru0403@gmail.com](mailto:satoru0403@gmail.com)

33 Kosuke Fujita

34 <sup>a)</sup> Human Health Sciences, Graduate School of Medicine, Kyoto University,

35 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.

36 Telephone: +81-75-751-3964

37 Email: [fujita.kousuke.68m@st.kyoto-u.ac.jp](mailto:fujita.kousuke.68m@st.kyoto-u.ac.jp)

38 Jun Umehara

39 <sup>a)</sup> Human Health Sciences, Graduate School of Medicine, Kyoto University,

40 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.

41 Telephone: +81-75-751-3964

42 Email: [umehara.jun.77z@st.kyoto-u.ac.jp](mailto:umehara.jun.77z@st.kyoto-u.ac.jp)

43 Sayaka Nakao

44    <sup>a)</sup> Human Health Sciences, Graduate School of Medicine, Kyoto University,

45    53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.

46    Telephone: +81-75-751-3964

47    E-mail: nakao.sayaka.37s@st.kyoto-u.ac.jp

48    Satoko Ibuki

49    <sup>a)</sup> Human Health Sciences, Graduate School of Medicine, Kyoto University,

50    53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.

51    Telephone: +81-75-751-3951

52    E-mail: [tokumitsu.satoko.6c@kyoto-u.ac.jp](mailto:tokumitsu.satoko.6c@kyoto-u.ac.jp)

53    ***Key words:*** shear wave elastography, semitendinosus, semimembranosus, biceps

54    femoris

55    ***Acknowledgement***

56    This work was supported by Grant-in-Aid for Scientific Research (B) 15H03043.

57

58

59

60 **ABSTRACT**

61 The aims of this study were to investigate the effects of a 4-week intervention of static  
62 stretching (SS) on muscle hardness of the semitendinosus (ST), semimembranosus  
63 (SM), and biceps femoris (BF) muscles. Shear elastic modulus was measured by using  
64 ultrasound shear wave elastography as the index of muscle hardness. Thirty healthy men (age,  
65  $22.7 \pm 2.2$  years) volunteered for this study and were randomly assigned to the SS-  
66 intervention group (n = 15) or the control group (n = 15). Subjects in the SS-  
67 intervention group received a 4-week stretch intervention for the hamstrings of their  
68 dominant leg. Shear elastic moduli of the hamstrings were measured at initial evaluation  
69 and after four weeks in both groups at a determined angle. In all muscles, the shear  
70 elastic modulus decreased significantly after SS intervention. The percentage change in  
71 the shear elastic modulus from the value at initial evaluation to after four weeks  
72 intervention was greatest in the SM. These results suggest that SS-intervention has  
73 chronic effects on reducing hardness of the hamstring muscle components, especially  
74 the SM muscle.

75 **Key words:** shear wave elastography, semitendinosus, semimembranosus, biceps  
76 femoris

77    **INTRODUCTION**

78        Static stretching (SS) is often used to increase flexibility of the hamstrings. There are  
79        many studies investigating the acute (Magnusson SP, Aagaard P, Simonsen E, Bojsen-  
80        Moller F, 1998; Magnusson SP, Aagaard P, Nielson JJ, 2000; Matsuo et al. 2013) or  
81        chronic (Ben & Harvey 2010; Marshall PW, Cashman A, Cheema BS, 2011) effects of  
82        SS on the flexibility of the hamstrings. Most previous studies (Magnusson SP,  
83        Simonsen EB, Aagaard P, Sorensen H, Kjaer M, 1996; Folpp H, Deall S, Harvey LA,  
84        Gwinn T, 2006; Ylinen et al. 2009; Ben & Harvey 2010) reported that the joint range  
85        of motion increased, but the stiffness of the muscle-tendon unit MTU and passive  
86        torque did not decrease after a few weeks of SS intervention on the hamstrings. On the  
87        other hand, a recent study (Marshall et al. 2011) showed that stiffness of the hamstrings  
88        decreased after a 4-week SS intervention, which is inconsistent with previous studies.

89        Marshall et al. reported that the reason for the change in passive torque after their  
90        stretch intervention may be due to high volume of the exercise (30 sec×4 sets、4  
91        exercises/session, self-stretching, 12 to 15 min/session). However, the interventions in  
92        studies reporting no change in passive torque are not necessarily low in volume, for  
93        instance, Ben & Harvey (2010) adopted interventions of 30 min/day, and Folpp et al.  
94        (2006) of 20 min/day. Since previous studies using passive torque as an index had

shown conflicting results, we attempted to evaluate the effect of stretching from another viewpoint, focusing solely on muscles by measuring shear elastic modulus.

One of the major concerns is that the effect of SS on individual muscles of the hamstrings cannot be determined by measuring the effect of the stiffness of MTU and passive torque during stretching. The passive torque measured during knee extension or hip flexion is a sum of the passive resistance of all the properties including the whole hamstring muscles and the surrounding structures. With the development of the method evaluating the mechanical properties of the muscle using ultrasound shear wave elastography, shear elastic modulus, which is an index of muscle hardness of each muscle composing the hamstrings can be evaluated separately(Nakamura M, Ikezoe T, Tokugawa T, Ichihashi N, 2015). While shear wave elastography measures the muscle hardness in the direction perpendicular to the line of force during contractile loading, this value is found to be correlated to muscle stiffness along the line of the muscle-tendon unit. (Nakamura et al. 2014). Therefore, in this paper, shear elastic modulus is used as a value to estimate the muscle hardness or extensibility.

High injury rate of the hamstring muscle strain during sports activity is found



113 (Brooks JH, Fuller CW, Kemp SP, Reddin DB, 2006; Feeley et al. 2008; Ekstrand J,  
114 Hagglund M, Walden M, 2011), and there is a relevance between the injury cite and the  
115 characteristics of the sport. For example, sprinters have a high risk of straining the biceps  
116 femoris(BF) muscle (Verrall GM, Slavotinek JP, Barnes PG, Fon GT, 2003; Koulouris G,  
117 Connell DA, Brukner P, Schneider-Kolsky M, 2007). On the other hand, dancers and  
118 ballerinas have a high risk of straining for the semimembranosus(SM) muscle (Askling  
119 CM, Tengvar M, Saartok T, Thorstensson A, 2008). Investigating the effects of SS on  
120 individual muscles of the hamstrings would be valuable for the understanding and  
121 preventing of muscle strain in the components of the hamstrings. Our previous study  
122 using ultrasound shear wave elastography (Umegaki et al. 2015) reported that the  
123 hardness of individual muscles comprising the hamstrings is decreased immediately after  
124 5 min of SS with hip flexion and knee extension, and that the SS maneuver may be most  
125 effective for the SM muscle. There is also a study which measured the immediate change  
126 in shear modulus of each hamstring muscles before and after 5 times of 90 seconds static  
127 stretching (Miyamoto, N., Hirata, K.,Kanehisa, H.,2015). This study found that hardness  
128 is reduced in SM and semitendinosus (ST) muscles, but not in the BF from hamstring  
129 stretching by passive knee extension maneuver. There are, however, no studies  
130 investigating the effects of a few-week SS program on the individual muscles of the

hamstrings. The originality of this study is that the effect of stretching intervention was evaluated using shear elastic modulus as an index of muscle hardness and that we measured each component of the hamstrings separately, which was not found in previous studies.

The aims of this study were to investigate the effects of a 4-week intervention of SS on muscle hardness of the ST, SM, and BF muscles, and to examine the differences in the chronic effects between these muscles using shear elastic modulus measured by ultrasound shear wave elastography.

## METHODS

### Subjects

Thirty healthy men (age  $22.7 \pm 2.2$  years; height  $171.4 \pm 4.6$  cm; weight  $63.7 \pm 8.5$  kg) participated in this study. The subjects were non-athletes who were not involved in any kind of regular stretching activity. Subjects with a history of neuromuscular disease, or musculoskeletal injury involving their lower limbs, were excluded from this study. The purpose and procedures were explained to all subjects, following which written informed consent was obtained. This study was approved by the ethics committee of Kyoto University Graduate School and the Faculty of Medicine (E-1524).

\*\*\*Table 1 near here\*\*\*

150

## 151 **Experimental protocol**

152 Subjects were randomly assigned to the SS-intervention group (n = 15) or the control  
153 group (n = 15). Subjects in the SS-intervention group performed a 4-week SS program  
154 for the hamstrings of their dominant leg, while subjects in the control group received no  
155 intervention. The dominant leg was defined as the leg with which the subject used to kick a ball.  
156 The subject did not participate in any other exercise during the intervention period. Shear elastic  
157 modulus of the hamstrings at 90° hip flexion and 45° knee flexion were measured  
158 before and after the 4-week intervention in both groups to evaluate muscle hardness. The  
159 subjects were instructed to remain relaxed during each of the measurements. The  
160 experimental protocol is shown in Fig. 1. To avoid the acute effects of SS, all  
161 measurements in the SS-intervention group were performed at least 24 h after the last SS  
162 session (Nakamura M, Ikezoe T, Takeno Y, Ichihashi N, 2012).

163 \*\*\*Figure 1 near here\*\*\*

164

## 165 **SS protocol**

166 The SS-intervention group performed the 4-week SS program using a dynamometer  
167 (Biodex system 4.0, Biodex Medical Systems Inc., USA). The subjects lay in supine

position, with their pelvis inclined anteriorly by placing a wedge between the pelvis and the bed. The dominant lower leg was attached to the dynamometer, with the hip angle fixed at 90° flexion. The knee was then passively extended to the angle that the subjects could tolerate without pain or discomfort, which was determined for each subjects before intervention. This form of stretching was chosen for the reproducibility of the stretch intensity, stretching position with the pelvis stabilised, and for the consistency with the measurement position. In our previous study (Umegaki et al.2015), we reported that the shear elastic modulus of individual muscles of the hamstrings decreased after 5 min of SS. Therefore, in this study, SS of 5 min was undertaken three times per week for four weeks.

#### **Measurement of shear elastic modulus**

Shear elastic modulus of the ST, SM, and BF muscle bellies in the dominant leg was measured using ultrasound shear wave elastography (Aixplorer; SuperSonic Imagine, Axi-en-Provence, France). The measurement sites were defined as the midpoint of the femur from the greater trochanter to the medial epicondyle for the ST and SM muscles, and to the lateral epicondyle of the femur for the BF muscle. These anatomical points

186 were confirmed by palpation and B-mode images. An ultrasound transducer (50 mm long  
187 SL-15-4 liner ultrasound transducer) was positioned on the measurement sites, parallel to  
188 the direction of the muscle fibers, which was confirmed by tracing several fascicles  
189 without interruption across the B-mode image. For each muscle, images were taken after  
190 the transducer was held at the measurement cite for around 5 s in order to confirm that  
191 the shear wave elastography(SWE) in the region of interest(ROI) showed stable color  
192 distribution. The measurements were taken twice for each muscle, and the mean values were used  
193 for statistical analysis. The region of interest was set near the centre of the muscle belly image, and  
194 the mean shear wave propagation speed (m/s) of an 11mmdiameter circle set near the centre of the  
195 ROI was automatically calculated. The accuracy of the measurement showed excellent reliability. ICC  
196 (1.1) and CV for ST, SM, and BF were 0.985 (1.8%), 0.971 (1.9%), and 0.983 (1.6%), respectively.

197 In the supine position, shear elastic modulus of the ST, SM, and BF muscles was  
198 measured at 90° hip flexion and 45° knee flexion. The shear elastic modulus was  
199 measured in <10 s for each measurement to avoid effects on muscle flexibility.  
200 Measurement of each component was performed in random order to exclude any effects  
201 of the measurement order. Each component was measured twice, and the mean values  
202 were used in the statistical analysis. None of the subjects expressed discomfort or pain,  
203 and the shear elastic modulus values did not reach the upper limit value during the

204 measurement.

205

## 206 **Inter-day reliability of the measurements**

207 To determine inter-day reliability of the measurements of the shear elastic modulus,  
208 measurements were taken in seven healthy men (age,  $23.6 \pm 1.8$  years; height,  $173.4 \pm$   
209  $3.7$  cm; weight,  $68.4 \pm 7.2$  kg) once each on two different days.

210

## 211 **A priori sample size calculation**

212 We calculated the sample size needed for split-plot analysis of variance (ANOVA)  
213 ( $\alpha$  error = 0.05, power = 0.80, effect size = 0.4 [large]), and the requisite number of  
214 subjects for this study was 14 in each group. We chose a large effect size on the basis of  
215 previous studies (Marshall et al. 2011; Akagi & Takahashi 2013).

216

## 217 **Statistical analysis**

218 Statistical analyses were performed using SPSS (version 18.0, SPSS Japan INC.,  
219 Tokyo, Japan). The inter-day reliability of the measurements was assessed using the  
220 intraclass correlation coefficient (ICC). Differences between the SS-intervention group  
221 and the control group, with regard to subject characteristics and all outcomes at baseline,

were assessed using an unpaired t-test. For the shear elastic modulus of each muscle, split-plot ANOVA using two factors (groups [the SS-intervention group and the control group] and test time [before and after four weeks]) was used to analyze interaction effects. When a significant interaction was observed, a paired t-test was used to determine the differences between the value at baseline and after four weeks in both the SS-intervention group and the control group. For shear elastic modulus in the SS-intervention group, two-way repeated measures ANOVA using two factors (test time [before and after four weeks] and muscles [ST, SM, and BF muscles]) was used to analyze interaction effects. When a significant interaction was observed, the Bonferroni's post-hoc test was used to compare the difference in the percentage change of shear elastic modulus between muscles. Percentage change of shear elastic modulus was calculated using the following equation:

$$\text{Percentage change (\%)} = (\text{at baseline} - \text{after four weeks}) / \text{at baseline} \times 100.$$

Differences were considered statistically significant at an alpha level of 0.05.

## RESULTS

### Characteristics of the subjects

No participants withdrew from the study and all subjects completed the SS program. Therefore, all data of the SS-intervention group (n = 15) and the control group (n = 15)

were used for statistical analysis. The characteristics of the subjects are shown in Table 1. There were no statistically significant differences between groups in the measured characteristics.

### **Inter-day reliability of the measurements**

The ICC(1,1)s of inter-day measurements are shown in Table 2. The ICC(1,1) ranged from 0.818 to 0.959 for the shear elastic modulus of each muscle. Since ICC value of <0.40 is generally considered as poor reliability, 0.40-0.75 as moderate to good, and >0.75 as excellent reliability (Leong et al., 2013), the results showed excellent reliability.

\*\*\*Table 2 near here\*\*\*

### **Shear elastic modulus**

The shear elastic modulus values are shown in Table 3. There were no significant group differences in shear elastic modulus of any of the muscles at baseline. Split-plot ANOVA indicated a significant interaction in all muscles (ST:  $F(1,28) = 7.2$ ,  $p = 0.01$ ; SM:  $F = 30.2$ ,  $p < 0.01$ ; BF:  $F(1,28) = 10.1$ ,  $p < 0.01$ ). In the SS-intervention group, shear elastic modulus of all the muscles significantly decreased after the intervention. In the control group, there were no changes in shear elastic modulus for any of the muscles.



258 \*\*\*Table 3 near here\*\*\*

259 As for the percentage change in the shear elastic modulus in the SS-intervention  
260 group, two-way ANOVA indicated a significant interaction ( $F = 17.6$ ,  $p < 0.01$ ). The  
261 Bonferroni's post-hoc test indicated that the percentage change in the shear elastic  
262 modulus of the SM muscle was significantly higher than that of the ST and BF muscles  
263 (Fig. 2).

264 \*\*\*Figure 2 near here\*\*\*

## 265 **DISCUSSION**

266 The results of this study showed that shear elastic modulus of the ST, SM, and BF  
267 muscles decreased after the 4-week SS intervention, which suggests that the SS  
268 program reduced the hardness of all muscles comprising the hamstrings. Although past  
269 studies (Magnusson et al. 1996; Folpp et al. 2006; Ylinen et al. 2009; Ben & Harvey  
270 2010;) have investigated the chronic effects of SS on the MTU of the hamstrings; to the  
271 best of our knowledge, this is the first report investigating the chronic effects of SS on  
272 the individual muscles comprising the hamstrings using ultrasound shear wave  
273 elastography. Our results contradict to the majority of the previous studies on  
274 stretching of the hamstring muscles that reported no change in stiffness (Magnusson et  
275 al. 1996; Folpp et al. 2006; Ylinen et al. 2009; Ben & Harvey 2010). The intervention

276 used in our study is not greater in volume compared to the studies reporting no change.  
277 Therefore, we assume that the difference may be due to the index used: shear elastic  
278 modulus or passive torque, the former focusing on the muscle itself, and the latter  
279 focusing on the whole muscle-tendon unit and the surrounding structure. The current  
280 study indicates that 4 weeks of stretching intervention for the hamstrings increases  
281 flexibility in the hamstrings muscle, especially in SM.

282 In this study, the shear elastic modulus as the index of the individual muscle  
283 hardness, reduced in all ST, SM and BF after the SS intervention. Based on the previous  
284 study indicating the positive correlation of the shear elastic modulus and passive torque  
285 during stretching of the muscle (Nakamura et al, 2014), the current result suggested that  
286 the 4 weeks SS intervention improved the flexibility of the hamstrings.

287 Our results showed that the shear elastic modulus of the ST, SM, and BF muscles  
288 decreased after the SS intervention, which was similar to a previous study (Akagi &  
289 Takahashi 2013) investigating the chronic effects of SS on the hardness of the medial  
290 and lateral gastrocnemius muscle. Therefore, our SS protocol (5 min of SS, three times  
291 per week for four weeks) may be effective for reduced hardness of all muscles comprising  
292 the hamstrings.

293 As for differences in the effects of the SS intervention between the muscles, the

294 percentage change in shear elastic modulus of the SM muscle was significantly greater  
295 than that of the ST and BF muscles. These results support our previous study (Umegaki  
296 et al.2015), which reports that the acute effect of SS is greatest in the SM muscle among  
297 the hamstring muscles. In our previous study (Umegaki et al.2015), it was estimated that  
298 passive tension applied to the SM muscle was highest during this SS maneuver, therefore;  
299 the chronic effect of the SS intervention might also be greatest in the SM muscle.

300         Poor flexibility of the hamstrings increases the risk of muscle strain (Witvrouw  
301 E, Danneels L, Asselman P, D'Have T, Cambier D, 2003; Bradley & Portas 2007). Our  
302 results indicated that SS program reduced the hardness of the individual muscles  
303 comprising the hamstrings. Therefore, the SS intervention protocol may be useful alter  
304 the incidence of hamstrings muscle strain. In particular, considering that the effect of the  
305 SS intervention was greatest in the SM muscle, the SS intervention protocol in this study  
306 may be more effective for preventing muscle strain in dancers and ballerinas, who are at  
307 high risk of straining the SM muscle (Askling CM, Tengvar M, Saartok T, Thorstensson  
308 A, 2008). We, however, did not investigate whether this SS intervention protocol is  
309 effective for preventing hamstring muscle strain, and therefore further research is still  
310 required to clarify the effect of the SS program on injury prevention.

311         This study also has some limitations that remain to be addressed. First, because

the subjects in the present study were healthy young men, it is unknown whether the SS protocol used in this study could be effective for women, the elderly, or subjects with a history of hamstring muscle strain. Second, it could not be demonstrated whether passive tension applied to the SM muscle among the hamstrings was the highest during this SS maneuver, because shear elastic modulus during SS was not measured. Furthermore, the experimenter who performed the measurements and data analysis was not masked to the nature of the study design.

## 5 CONCLUSIONS

Our results indicate that the SS intervention significantly decreased the shear elastic modulus of all the hamstring muscles. The percentage change in the shear elastic modulus after four weeks was greatest in the SM. Since poor flexibility of the hamstrings is known to increase the risk of muscle strain (Witvrouw et al. 2003; Bradley & Portas 2007), our results indicated that SS program may contribute to alter the risk of injury.

## ACKNOWLEDGEMENT

This work was supported by Grant-in-Aid for Scientific Research (B) 15H03043.

## 331 REFERENCES

- 332 Akagi R, & Takahashi H. (2013). Effect of a 5-week static stretching program on  
333 hardness of the gastrocnemius muscle. *Scandinavian journal of medicine & science in*  
334 *sports*, 24,950-957. doi: 10.1111/sms.12111.
- 335 Askling CM, Tengvar M, Saartok T, Thorstensson A.(2008). Proximal hamstring strains  
336 of stretching type in different sports - Injury situations, clinical and magnetic resonance  
337 imaging characteristics, and return to sport. *Am J Sport Med*, 36, 1799-1804. doi:  
338 10.1177/0363546508315892
- 339 Ben M, Harvey LA. (2010). Regular stretch does not increase muscle extensibility: a  
340 randomized controlled trial. *Scandinavian journal of medicine & science in sports*, 20,  
341 136-144. doi: 10.1111/j.1600-0838.2009.00926.x.
- 342 Bradley PS, Portas MD. (2007).The relationship between preseason range of motion  
343 and muscle strain injury in elite soccer players. *Journal of Strength and Conditioning*  
344 *Research*, 21, 1155-1159.
- 345 Brooks JH, Fuller CW, Kemp SP, Reddin DB. (2006) Incidence, risk, and prevention of  
346 hamstring muscle injuries in professional rugby union. *The American journal of sports*  
347 *medicine*, 34, 1297-1306.
- 348 Congdon R, Bohannon R, Tiberio D. (2005) Intrinsic and imposed hamstring length

349 influence posterior pelvic rotation during hip flexion. *Clinical biomechanics*, 20: 947-  
350 951.

351 Eby, S. F., Cloud, B. A., Brandenburg, J. E., Giambini, H., Song, P., Chen, S.,  
352 LeBrasseur NK., An, K. N. (2015). Shear wave elastography of passive skeletal muscle  
353 stiffness: influences of sex and age throughout adulthood. *Clin Biomech (Bristol, Avon)*,  
354 30, 22-27. doi: 10.1016/j.clinbiomech.2014.11.011

355 Ekstrand J, Hagglund M, Walden M.(2011). Injury incidence and injury patterns in  
356 professional football: the UEFA injury study. *Br J Sports Med*, 45, 553-558. doi:  
357 10.1136/bjsm.2009.060582.

358 Feeley BT, Kennelly S, Barnes RP, Muller MS, Kelly BT, Rodeo SA, Warren RF.  
359 (2008). Epidemiology of National Football League training camp injuries from 1998 to  
360 2007. *The American journal of sports medicine*, 36, 1597-1603. doi:  
361 10.1177/0363546508316021.

362 Folpp H, Deall S, Harvey LA, Gwinn T. (2006). Can apparent increases in muscle  
363 extensibility with regular stretch be explained by changes in tolerance to stretch?  
364 *Australian Journal of Physiotherapy*, 52, 45-50.

365 Koulouris G, Connell DA, Brukner P, Schneider-Kolsky M. (2007). Magnetic resonance  
366 imaging parameters for assessing risk of recurrent hamstring injuries in elite athletes.

- 367     *The American journal of sports medicine*, 35: 1500-1506.
- 368     Lee, S. S., Spear, S., & Rymer, W. Z. (2015). Quantifying changes in material properties  
369     of stroke-impaired muscle. *Clin Biomech (Bristol, Avon)*, 30, 269-275. doi:  
370     10.1016/j.clinbiomech.2015.01.004
- 371     Leong, H.T., Ng, G.Y., Leung, V.Y., Fu, S.N.(2013). Quantitative estimation of muscle  
372     shear elastic modulus of the upper trapezius with supersonic shear imaging during arm  
373     positioning. *PLoS One*, 25:8 e67199. doi:10.1371/journal.pone.0067199
- 374     Magnusson SP, Simonsen EB, Aagaard P, Sorensen H, Kjaer M. (1996). A mechanism  
375     for altered flexibility in human skeletal muscle. *J Physiol-London*, 497, 291-298.
- 376     Magnusson SP, Aagard P, Simonsen E, Bojsen-Moller F. (1998). A biomechanical  
377     evaluation of cyclic and static stretch in human skeletal muscle. *International journal of*  
378     *sports medicine*, 19, 310-316.
- 379     Magnusson SP, Aagaard P, Nielson JJ. (2000). Passive energy return after repeated  
380     stretches of the hamstring muscle-tendon unit. *Medicine and science in sports and*  
381     *exercise*, 32, 1160-1164.
- 382     Marshall PW, Cashman A, Cheema BS. (2011). A randomized controlled trial for the  
383     effect of passive stretching on measures of hamstring extensibility, passive stiffness,

- 384 strength, and stretch tolerance. *Journal of science and medicine in sport / Sports*  
385 *Medicine Australia*, 14, 535-540. doi: 10.1016/j.jsams.2011.05.003.
- 386 Matsuo S, Suzuki S, Iwata M, Banno Y, Asai Y, Tsuchida W, Inoue T.(2013). Acute  
387 effects of different stretching durations on passive torque, mobility, and isometric  
388 muscle force. *Journal of strength and conditioning research / National Strength &*  
389 *Conditioning Association*, 27, 3367-3376. doi: 10.1519/JSC.0b013e318290c26f.
- 390 Miyamoto, N.,Hirata, K., Kanehisa, H.(2015). Effects of hamstring stretching on  
391 passive muscle stiffness vary between hip flexion and knee extension maneuvers. *Scand*  
392 *J Med Sci Sports*, Dec 16. doi: 10.1111/sms.12620.
- 393 Morse CI, Degens H, Seynnes OR, Maganaris CN, Jones DA. (2008). The acute effect  
394 of stretching on the passive stiffness of the human gastrocnemius muscle tendon unit.  
395 *The Journal of physiology*, 586, 97-106.
- 396 Nakamura M, Ikezoe T, Takeno Y, Ichihashi N. (2011). Acute and prolonged effect of  
397 static stretching on the passive stiffness of the human gastrocnemius muscle tendon unit  
398 in vivo. *Journal of orthopaedic research : official publication of the Orthopaedic*  
399 *Research Society*, 29, 1759-1763. doi: 10.1002/jor.21445.
- 400 Nakamura M, Ikezoe T, Takeno Y, Ichihashi N. (2012). Effects of a 4-week static stretch  
401 training program on passive stiffness of human gastrocnemius muscle-tendon unit in



402 vivo. *European journal of applied physiology*, 112, 2749-2755. doi: 10.1007/s00421-  
403 011-2250-3.

404 Nakamura M, Ikezoe T, Tokugawa T, Ichihashi N. (2014). Acute effects of stretching  
405 on passive properties of human gastrocnemius muscle-tendon unit: Analysis of  
406 differences between hold-relax and static stretching. *J Sport Rehabil*, 24, 286-92. doi:  
407 10-1123/jsr.2014-0164.

408 Umegaki H, Ikezoe T, Nakamura M, Nishishita S, Kobayashi T, Fujita K, ...Ichihashi  
409 N. (2015). Acute effects of static stretching on the hamstrings using shear elastic  
410 modulus determined by ultrasound shear wave elastography: Differences in flexibility  
411 between hamstring muscle components. *Manual therapy*, 20, 610-3. doi:  
412 10.1016/j.math.2015.02.006.

413 Verrall GM, Slavotinek JP, Barnes PG, Fon GT. (2003). Diagnostic and prognostic value  
414 of clinical findings in 83 athletes with posterior thigh injury: comparison of clinical  
415 findings with magnetic resonance imaging documentation of hamstring muscle strain.  
416 *The American journal of sports medicine*, 31, 969-973.

417 Witvrouw E, Danneels L, Asselman P, D'Have T, Cambier D.(2003). Muscle flexibility  
418 as a risk factor for developing muscle injuries in male professional soccer players - A  
419 prospective study. *Am J Sport Med*, 31, 41-46.

- 420 Ylinen J, Kankainen T, Kautiainen H, Rezasoltani A, Kuukkanen T, Hakkinen A.(2009).
- 421 Effect of Stretching on Hamstring Muscle Compliance. *Journal of Rehabilitation*
- 422 *Medicine*, 41, 80-84.
- 423

424 Table 1. Characteristics of the subjects

	SS-intervention group	Control group
Age (years)	22.5 ± 2.9	22.9 ± 1.2
Height (cm)	171.9 ± 5.3	171.0 ± 3.8
Weight (kg)	65.7 ± 8.4	61.7 ± 8.3

425 Values are expressed as mean ± SD (standard deviation).

426

427

428 Table 2. Inter-day reliability of the measurements

	ICC(1,1)	CV(%)
Shear elastic modulus of ST	0.938	4.2
Shear elastic modulus of SM	0.818	3.9
Shear elastic modulus of BF	0.959	2.1

429 ST: semitendinosus, SM: semimembranosus, BF: biceps femoris, ICC: intraclass

430 correlation coefficient, CV: coefficient of variation

431 Table 3. The effects of SS on shear elastic modulus

	SS-intervention group		Control group	
	baseline	after four weeks	baseline	after four weeks
ST (kPa)	54.5 ± 14.4	47.5 ± 12.5**	48.7 ± 17.2	48.2 ± 14.3
SM (kPa)	126.6 ± 25.4	99.4 ± 18.9**	120.7 ± 39.5	118.0 ± 25.4
BF (kPa)	95.9 ± 28.0	82.3 ± 22.5**	85.6 ± 25.5	83.6 ± 20.5

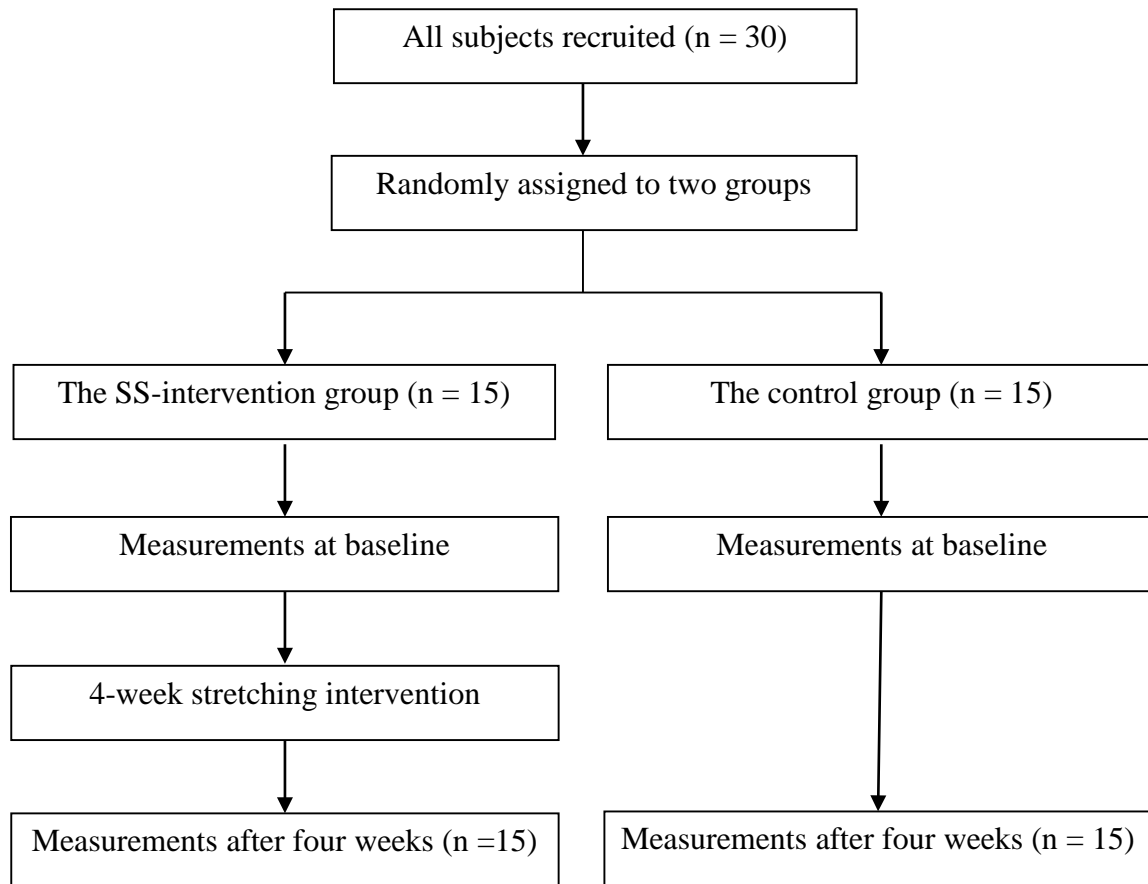
432 Values are expressed as mean ± SD (standard deviation).

433 \*\* p < 0.01; significant difference compared with baseline.

434 ST: semitendinosus, SM: semimembranosus, BF: biceps femoris

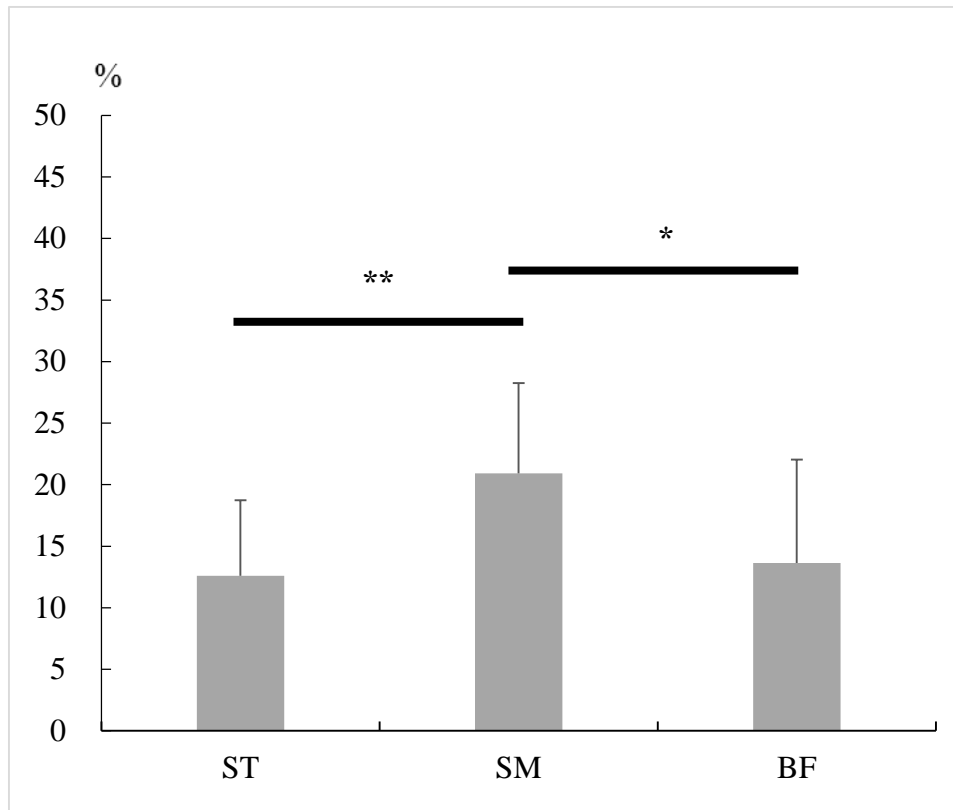
435

436 Figure 1. Flow chart of the experimental protocol



437

438 Figure 2. Differences in the percentage change of shear elastic modulus between muscles



439

440 Values are expressed as mean  $\pm$  SD (standard deviation).

441 \*  $p < 0.05$

442 \*\*  $p < 0.01$

443 ST: semitendinosus, SM: semimembranosus, BF: biceps femoris

444